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# U.S. NAVAL MEDICAL RESEARCH LABORATORY



Submarine Base, New London, Conn.

Vol. XX, No. 14

REPORT NO. 360

15 Sept 1961

UNCLASSIFIED

EFFECT OF PROLONGED EXPOSURE TO 15% CARBON DIOXIDE  
ON CALCIUM AND PHOSPHORUS METABOLISM

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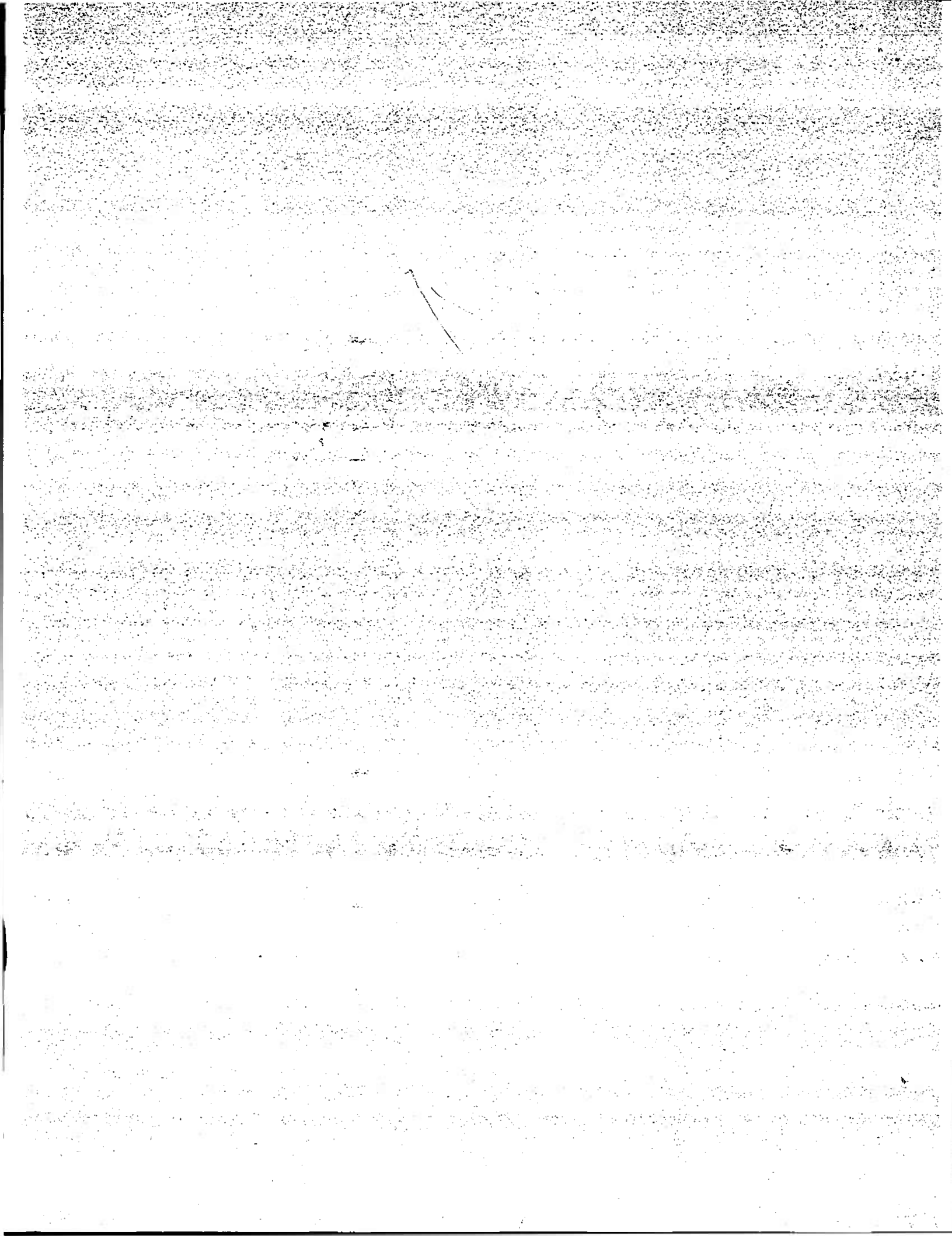
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Bureau of Medicine and Surgery, Navy Department  
Research Project MR005.14-3002-1.05



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1961, v107, 355-359

### Effect of Prolonged Exposure to 15% CO<sub>2</sub> on Calcium and Phosphorus Metabolism.\* (26624)

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Brown and Prasad(1) reported slight increases in plasma calcium and inorganic phosphorus, associated with a fall in ultrafiltrable Ca, during acute exposure of dogs to high CO<sub>2</sub> concentrations (30-40% CO<sub>2</sub>). Nichols, Schaefer and Carey(2) found in men during a chronic slight respiratory acidosis (exposure to 1.5% CO<sub>2</sub> for 42 days) a decrease in plasma calcium and increase in inorganic phosphorus during the period of uncompensated respiratory acidosis. In experiments reported here, calcium and phosphorus metabolism was studied during a period of chronic respiratory acidosis induced by exposure to 15% CO<sub>2</sub> in guinea pigs.

**Methods.** Male guinea pigs of the Hartley strain weighing between 400 and 550 g were exposed to 15% CO<sub>2</sub> in 21% O<sub>2</sub> for periods up to 73 days. Two groups of animals were studied after recovery on air for 1 and 11 days respectively following exposure to 15% CO<sub>2</sub> for 7 days. In a second series of experiments urine was collected daily in metabolism cages prior, during, and after exposure to CO<sub>2</sub>, from 4 guinea pigs. The number of animals used in each experimental group is listed in Table I. Blood samples were taken under anaerobic conditions through heart punctures after the animal was stunned by a blow at the base of the skull. pH was measured in a constant temperature waterbath at 37°C by a model G Beckman pH meter using hypodermic glass electrodes. Whole blood CO<sub>2</sub> content was determined with the Van Slyke apparatus. Experimental chambers allowed good control of temperature and humidity (78°F ± 2° and 65-75% humidity). Hematocrit was measured using the microcapillary method. Blood plasma calcium and urinary calcium were determined by the method of Clarke and Collip, a modification

of the procedure of Kramer and Tisdall(3). Blood calcium data after one hour exposure were obtained using the EDTA method of Munson *et al.*(4). The inorganic phosphorus in blood and urine was measured by the method of Fiske and Subbarow as modified by Roe and Whitmore(5). Plasma protein was measured using the method of Wolfson, Cohn, Calvary and Schiba(6). The albumin/globulin ratio was determined in a limited number of blood samples in animals exposed to CO<sub>2</sub> for various time periods. Data on ultrafiltrable calcium were obtained by the method of Toribara *et al.*(7).

**Results.** Table I presents results on plasma pH, calcium, inorganic phosphorus, ultrafiltrable calcium and plasma protein. The pH shows a marked drop after one hour and one day of exposure to 15% CO<sub>2</sub>, and increases to a level somewhat below normal but not significantly different after the second to third day. This indicates that after 2-3 days a compensation of the respiratory acidosis is reached. This is supported by other data on urine pH, plasma and urine electrolytes reported elsewhere(8). Plasma calcium rises slightly and inorganic phosphorus shows a small decrease after one day of exposure to 15% CO<sub>2</sub>. On the second to third day and thereafter plasma calcium increases and plasma phosphorus decreases significantly. After 4-7 days and after 20 days of exposure calcium level is still elevated and phosphorus level remains lowered. Recovery on air for one day after exposure to 15% CO<sub>2</sub> for 7 days does not change this status. After 11 days of recovery, inorganic phosphorus has returned to initial levels while plasma calcium is somewhat lower than control values.

Ultrafiltrable calcium increases slightly during the first phase of CO<sub>2</sub> exposure and is significantly higher after 4-7 days of exposure and after one day recovery following

\* This investigation was supported in part by O.N.R.

† With the technical assistance of J. R. Cassidy.

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**TABLE I.** Effect of Prolonged Exposure to 15% CO<sub>2</sub> on Blood pH, Plasma Calcium, Inorganic Phosphorus, Ultrafiltrable Calcium and Total Protein.

		pH of blood	Plasma calcium, mg %	Plasma inorg. phos- phorus, mg %	Total pro- tein, g %	Ultrafiltrable calcium, mMol/l	Fraction of total calcium
Control	Mean	7.38	11.6	7.0	8.4	1.31	.45
	S.D.	.05	.6	1.1	.14	.22	.07
	No.	25	15	20	8	8	8
15% CO <sub>2</sub> 1 hr	Mean	7.07†	11.6	6.7	—	1.41	.54
	S.D.	.02	.6	.9	—	.10	.03
	No.	12	6	13	—	6	6
15% CO <sub>2</sub> 1 day	Mean	7.21†	12.0	6.8	8.7	1.40	.48
	S.D.	.08	.9	.9	.5	.30	.09
	No.	38	14	11	12	9	9
15% CO <sub>2</sub> 2-3 days	Mean	7.29†	12.9*	5.8†	8.5	—	—
	S.D.	.03	.6	.9	.9	—	—
	No.	25	13	17	8	—	—
15% CO <sub>2</sub> 4-7 days	Mean	7.35	12.1	5.6†	8.4	1.69†	.60*
	S.D.	.06	.6	.7	.3	.20	.05
	No.	34	14	8	13	10	10
15% CO <sub>2</sub> 20-73 days	Mean	7.34	13.2*	6.1†	8.6	—	—
	S.D.	.08	.6	.2	.25	—	—
	No.	12	13	10	4	—	—
7 day exp.							
15% CO <sub>2</sub> 1 hr rec.	Mean	—	11.6	—	—	1.58†	.65*
	S.D.	—	.5	—	—	.14	.09
	No.	—	7	—	—	4	4
15% CO <sub>2</sub> 1 day rec.	Mean	7.34*	12.4*	5.5†	—	1.64†	.50
	S.D.	.08	.4	.9	—	.24	.06
	No.	21	8	11	—	4	4
15% CO <sub>2</sub> 11 day rec.	Mean	7.37	10.9	6.9	8.0*	—	—
	S.D.	.06	.5	1.0	.4	—	—
	No.	23	6	8	5	—	—

\* Statistically significant from control values at the 5% level.

† " " " " " " " " 1% and greater.

7 days exposure. No changes in total protein were observed.

Fig. 1 shows measurements of daily urine volume, excretion of urinary calcium, and inorganic phosphorus obtained from a group of 4 guinea pigs prior to and during exposure to 15% CO<sub>2</sub>, and during a recovery period on air following exposure. Urinary volume increases slightly during the first day of CO<sub>2</sub> exposure and decreases during the following days of exposure. After 5-6 days of recovery, control values are reached. A rise above initial levels is noted during the period of 6-11 days recovery. During the first phase of uncompensated respiratory acidosis, calcium as well as phosphorus excretion is significantly increased. Calcium excretion falls during the second phase of CO<sub>2</sub> exposure be-

low control values, while phosphorus excretion shows a transitory decrease during the second and third day to initial levels followed

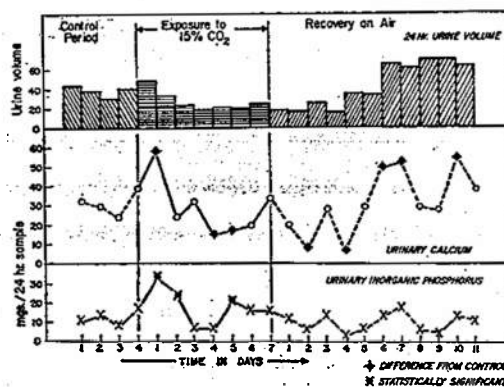


FIG. 1. Effect of prolonged exposure of guinea pigs to 15% CO<sub>2</sub> on 24 hour urine volume and urinary excretion of calcium and inorganic phosphorus.





FIG. 2a. Interstitial calcium deposits in the renal medulla of a guinea pig exposed to 15% CO<sub>2</sub> for 7 days. 2b. Intra tubular calcium deposit in the renal cortex of a guinea pig exposed to 15% CO<sub>2</sub> for 73 days. 2c. Calcium deposits in tubular basement membranes of the renal cortex of a guinea pig exposed to 15% CO<sub>2</sub> for 47 days.

by a second significant rise during the fifth day of the exposure period when a value of  $20.5 \pm 7.2$  was reached. Circled values indicate daily phosphorus and calcium excretion levels which differ from control values at a 5% level of confidence and greater.

**Histopathologic findings.** In connection with the evidence for changes in calcium and phosphorus metabolism occurring under chronic exposure to 15% CO<sub>2</sub> mention should be made of the finding of renal calcinosis in

these animals. Our series of 22 test animals and 4 controls showed a fairly irregular occurrence of renal calcifications, starting as early as 48 hours after beginning of exposure. The lesions were never severe, except in 2 animals which were exposed for more than one month. The localization and type of the lesions appeared not to follow rigid pattern. Three main types of calcification were noted: 1) interstitial deposits, usually confined to the medulla and often in immediate vicinity of the tip of the papilla (Fig. 2a); 2) intratubular deposits, commonly in the cortex or medulla close to the cortico-medullary junction. These were associated with changes in tubular epithelium (flattening, pyknosis of nuclei and basophilia of cytoplasm regeneration) as shown in Fig. 2b; 3) occasionally calcification originated in the tubular basement membranes, as shown in Fig. 2c, where the epithelium is lifted off its base and pushed into the lumen of the tubule. This lesion was located near the cortico-medullary junction.

**Discussion.** The fall in plasma inorganic phosphorus associated with a rise in plasma calcium found during chronic respiratory acidosis in guinea pigs represents a major difference between the reported increase in inorganic phosphorus in response to acute exposure to CO<sub>2</sub> for periods of 2-4 hours(1). The changes in plasma calcium and phosphorus observed in our experiments are similar to those produced by parathyroid stimulation or injection of parathyroid extract(9). The increased urinary phosphorus excretion during the first and fifth day of exposure to 15% CO<sub>2</sub> indicates 2 phases of increased parathyroid activity. The second phase, however, could not be detected in plasma calcium and phosphorus values since these data were not collected on every single day of exposure. Reported findings agree with results of a study by Stanmeyer, King, Scofield and Colby(10) on rats. They observed, on the basis of daily measurements during a 7 day period of exposure to 15% CO<sub>2</sub>, two phases at the end of the second and seventh day in which plasma calcium increased and plasma phosphorus decreased. Data obtained in 2 species of animals suggest development

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of an increased parathyroid activity during chronic exposure to CO<sub>2</sub> which apparently does not occur in acute CO<sub>2</sub> exposure.

A decrease in pH, as found in respiratory acidosis, is known to produce a decrease in calcium binding of proteins(11) and a rise in ionized and ultrafiltrable calcium(12,13). The average value of ultrafiltrable calcium (controls) obtained in our experiments, 1.31 mM/l serum, agrees with data reported in the literature on mean ultrafiltrable calcium in normal animals amounting to 1.31 mMol/l(13). However, the mean percentage of total calcium observed, 45%, is smaller than the 53.3% given by Prasad(13). Ultrafiltrable calcium increased only slightly during the period of CO<sub>2</sub> exposure in which decrease in pH was greatest, one hour and one day exposure, and more significantly after 7 days' exposure and after one hour and one day recovery on air following 7 days' exposure. In the latter conditions pH changes were rather small. The other factors known to influence calcium protein interactions, albumin/globulin ratio and total protein, were not significantly altered. The inorganic phosphorus, however, was much lower. These data seem to support the hypothesis of Brown and Prasad(1) that an increase in inorganic phosphate produces a decrease in ultrafiltrable calcium and *vice versa*, probably due to formation of some non-filtrable calcium phosphate compound, which is also in agreement with results of other investigations(14,15). However, definite proof of this theory would require measurements of ultrafiltrable inorganic phosphate and protein bound phosphate in the plasma *in vivo* under these experimental conditions. Although no fall in ultrafiltrable calcium was observed prior to the apparent parathyroid stimulation in the periods investigated, our data are not quite adequate to draw a conclusion as to whether CO<sub>2</sub> could be considered a stimulus to which the parathyroid gland responds directly.

Renal calcification in animals exposed intermittently and continuously for prolonged periods to increased concentrations of CO<sub>2</sub> have been reported by Meesen(16) and Zinck

(17). Anoxemia was excluded as being a factor in development of these lesions. Zinck(17) noted that the correlation between concentration of CO<sub>2</sub>, length of exposure, and severity of the renal lesion was poor, and that there was a great deal of individual variation. In extensive studies on experimental nephrocalcinosis, Goebel and Koburg(18) were able to produce renal calcium deposits by subjecting animals to respiratory insufficiency. The most frequent of the 3 types of renal calcification found in our experiments were the interstitial calcium deposits located in the inner medulla. A similar renal calcification located primarily interstitially in the medulla was reported in a case of parathyroid adenoma(19) while in another recent study(20) dealing with nephrocalcinosis in experimental hyperparathyroidism, interstitial calcification was described as "minimal".

The physiological data obtained in animals exposed to 15% CO<sub>2</sub> suggest that parathyroid stimulation plays a role in development of the reported renal lesions in chronic respiratory acidosis.

**Summary.** (1) During chronic respiratory acidosis, produced by prolonged exposure of guinea pigs to 15% CO<sub>2</sub>, plasma calcium was found to increase and plasma phosphorus to decrease significantly. (2) These changes appear to be related to an increased parathyroid activity as evidenced by an increased urinary phosphorus excretion. (3) Ultrafiltrable calcium increased only slightly during the period of uncompensated respiratory acidosis with the greatest pH changes, and rose much higher during the period of compensated respiratory acidosis where pH changes were smaller. During the latter period plasma inorganic phosphorus values were much lower, supporting the hypothesis of Brown(1) concerning the inverse relationship of ultrafiltrable calcium and inorganic phosphorus. (4) Histological studies showed a significant renal calcification in guinea pigs exposed for prolonged periods to 15% CO<sub>2</sub>.

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Received January 3, 1961. P.S.E.B.M., 1961, v107.